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Transport of a neonicotinoid pesticide, thiamethoxam, from artificial seed coatings☆

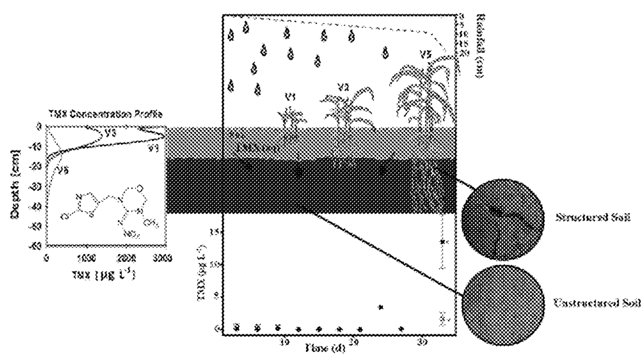
Jesse Radolinski [a,*], Junxue Wu [b], Kang Xia [a], Ryan Stewart [a]

[a] *Department of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute, 185 Ag Quad Lane, Blacksburg, VA 24061, USA*
[b] *Department of Applied Chemistry, China Agricultural University, No. 2 West Yuanmingyuan Road, Beijing, PR China*

HIGHLIGHTS

- Transport of neonicotinoid thiamethoxam (TMX) was quantified from corn seed coats.
- A column study with viable corn plants simulated field-realistic leaching.
- Evapo-concentration reduced and soil structure and plants enhanced TMX movement.
- TMX was transported from surface horizons in levels acutely toxic to aquatic life.

GRAPHICAL ABSTRACT



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ABSTRACT

Neonicotinoid insecticides coat the seeds of major crops worldwide; however, the high solubility of these compounds, combined with their toxicity to non-target organisms, makes it critical to decipher the processes by which they are transported through soils and into aquatic environments. Transport and distribution of a neonicotinoid (thiamethoxam, TMX) were investigated by growing TMX-coated corn seeds in coarse-textured and fine-textured soil columns (20 and 60 cm lengths). To understand the influence of living plants, corn plants were terminated in half of the columns (no plant treatment) and allowed to grow to the V5 growth stage (33 days of growth) in the other half (with plant treatment). TMX was analyzed in leachate 12 times over 33 days and in bulk soil after 8, 19, and 33 days of corn growth. All 20 cm columns leached TMX at levels exceeding the United States Environmental Protection Agency benchmark for aquatic invertebrates (17.5 µg L^{-1}). TMX migrated from seeds to adjacent bulk soil by the eighth day and reached deeper soil sections in later growth stages (e.g., 30–45 cm depth by Day 33). Fine-particle soils transported over two orders of magnitude more TMX than coarse-textured soils (e.g., 29.9 µg vs 0.17 µg, respectively), which was attributed to elevated evapotranspiration (ET) rates in the sandy soil driving a higher net retention of the pesticide and to structural flow occurring in the fine-textured soil. Living plants increased TMX concentrations at depth (i.e., 30–60 cm) compared to the no plant treatment, suggesting that corn growth may drive preferential transport of TMX from coated seeds. Altogether, this study showed that neonicotinoid seed coatings can be mobilized through soil leachate in concentrations considered acutely toxic to aquatic life.

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* Corresponding author at: 185 Ag Quad Lane, Blacksburg, VA 24061, USA.
E-mail address: jrad33@vt.edu (J. Radolinski).

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1. Introduction

Each year >20,000 t of neonicotinoids are produced (Codling et al., 2016) and applied to 140 + crops worldwide (Elbert et al., 2008; Main et al., 2015), with an estimated global market value of ~$2.6 billion (Goulson and Kleijn, 2013; Jeschke et al., 2011). Imidicloprid (IMD), thiamethoxam (TMX), and clothianidin (CLO) are the most common neonicotinoids, and are used extensively as seed coated insecticides. These three compounds have high selectivity for the insect nicotinic acetylcholine receptor, which makes them an effective pesticide against a broad spectrum of invertebrate pests while remaining relatively non-toxic to mammals (Jeschke and Nauen, 2007; Jeschke et al., 2011; Zalom et al., 2005). Despite their effectiveness as insecticides, neonicotinoids have been scrutinized for high toxicity to non-target invertebrate organisms and insectivorous birds (Douglas et al., 2015; Hallmann et al., 2014), including an association with significant declines in bee populations (Alix et al., 2009; Sanchez-Bayo and Goka, 2014; Sandrock et al., 2014; Whitehorn et al., 2012).

Globally, >60% of all neonicotinoids are applied via seed dressings (Goulson and Kleijn, 2013). Neonicotinoid seed coatings are advertised to provide continued protection from insect herbivory throughout the growing season, as the highly soluble compounds move into the root zone, enter the plant, and arm its aboveground tissue (Jeschke et al., 2011; Jones et al., 2014; Zalom et al., 2005). Because they are applied directly to the plant roots, seed coatings typically have lower amounts of active pesticides compared to spray applications (in terms of g ha^{-1}) and are therefore often considered to be more environmental friendly (Jeschke et al., 2011). However, only 2–20% of the pesticide is taken up by the target crop, with the remainder left in the soil environment where it may become transported into ground water and eventually incorporated into surface water systems (Sanchez-Bayo, 2014). Neonicotinoids can be applied as corn coatings at amounts up to 1.25 mg per seed. Using corn production (typical planting rate: 74,000 seeds ha^{-1}) as an example, an estimated 3300 t of neonicotinoids can be applied each year to the 36 million ha of maize in the United States (USDA, 2017). If only 20% of the neonicotinoids are taken up by the corn plants, then up to 2700 t of neonicotinoids may be mobilized in the surrounding environment. As a possible consequence, neonicotinoids are now detected in surface waters across North America (Hladik et al., 2014; Main et al., 2015; Schaafsma et al., 2015). In regions with intensive soybean and maize production, neonicotinoids have been detected in nearly all surface water bodies (Hladik et al., 2014; Schaafsma et al., 2015), with seed coatings identified as the most likely source. However, the linkage between seed coatings and the aquatic environment as not been directly assessed, and no work to date has identified or quantified the underlying mechanisms by which neonicotinoids are transported from agricultural fields.

Neonicotinoids have moderate to high leaching potential, with leaching patterns largely explained by the aqueous solubility of the compounds (Banerjee et al., 2008; Cox et al., 2001; Cox et al., 1997; Gupta et al., 2008; Katagi, 2013; Kurwadkar et al., 2014; Leiva et al., 2015; Oi, 1999). Leaching typically increases with soil water content; for example, the half-life of thiamethoxam (TMX) in soil columns decreased from 301 days to 46 days as soil water content increased from dry to near-saturated conditions, with degradation rates increasing with TMX concentration (Gupta et al., 2008). Because neonicotinoids are polar, highly soluble, and exhibit low affinity for soil mineral matrix, the partial equilibrium conditions provided by a rain storm may promote leaching via bulk flow or advection (Hu and Brusseau, 1996; Katagi, 2013; Kurwadkar et al., 2014; Oi, 1999).

Soil structural features, which arise as unconsolidated soil material arranges into a more stable hierarchy of aggregates, often form secondary pore networks that may affect solute transport. As soil water pressure increases during a rain event, flow can preferentially follow these structural pathways and bypass the soil matrix, where water is tightly held at more negative potentials (Jarvis, 2007). This non-equilibrium flow can result in pesticides being rapidly mobilized through the soil profile (FOCUS, 2001; Jarvis, 2007; Molz, 2015). For example, mass balances performed on the herbicide bentazone have showed that up to 8% of the applied dose can be lost through soil structural pathways, resulting in concentrations as high as 200 μg L^{-1} in tile drainage (FOCUS, 2001). Thus, it is important to understand if neonicotinoids can also be mobilized via flow through soil structural pathways.

The transport and distribution of pesticides in soil is also further complicated by the presence of plants. For instance, maize can apply suction forces well above 9.5 bars (Ionescu, 1969). For highly soluble compounds such as neonicotinoids, this plant-induced suction could translate to a vertical stratification in the soil profile, in which neonicotinoids can remain close to the soil surface under stable unsaturated conditions. On the other hand, roots may increase the size of soil structural pathways, which can cause increased preferential leaching (Bundt et al., 2000; Jørgensen et al., 2002). Such plant-related processes have not yet been well-studied in the context of pesticide transport.

The primary aim of this study was to quantify the transport of thiamethoxam (TMX) from coated corn seeds in fine-textured and coarse-textured soils, while also accounting for the roles of soil structure and viable plants. We hypothesize that: i) coarse-textured soil will transport more TMX than fine-textured soil with intact structural features, ii) structured soil will transport more than unstructured soil of the same texture, and iii) the presence of viable plants will result in less leaching of the pesticide from soil columns. Since seed coatings are the dominant form of neonicotinoid application for many crops, direct measurements of their movement from the seeds is an imperative step in assessing the overall environmental impact of this practice.

2. Materials and methods

2.1. Soil characterization

Soils were taken from pastures in New Kent and Whitehorne, VA. The New Kent soil was a Bojac series (Typic Hapludult) and coarse-textured (hereafter referred to as a "sand"). The Whitehorne soil was a Shottower series (Typic Paleudults) and a fine-textured, moderately structured soil (hereafter referred to as a "loam").

Intact soil cores (stainless steel, 2.5 cm × 5 cm) taken from the two sites were analyzed for bulk density [M L^{-3}] and saturated hydraulic conductivity (K_s) [L T^{-1}]. Samples were collected every 5 cm depth from the surface to 30 cm depth ($n = 6$ cores per depth increment). The 0–20 cm cores were considered to represent the A_p horizon and the 20–30 cm cores were considered to represent the B_t layer. K_s was determined using the falling head method with a UMS KSAT Benchtop Saturated Hydraulic Conductivity Instrument (UMS Inc.; Munich, Germany). K_s was determined per the manufacturer's recommendation as:

$$K_s = bL\left(\frac{A_{burette}}{A_{sample}}\right) \quad (1)$$

where $A_{burette}$ [L^2] is the cross-sectional area of the water column, A_{sample} [L^2] is the cross-sectional area of the sample, L is the length of the sample, and b is an exponent determined via curve-fitting between measured pressure head (h, starting at some initial pressure head h_0) and time:

$$h(t) = h_0 e^{-bt}. \quad (2)$$

Loose soil samples from the A_p (0–20 cm) and B_t (20–60 cm) horizons were air dried, sieved to 2 mm, and analyzed for pH, cation exchange capacity (CEC) [Mol M^{-1}], total organic carbon (TOC) [M M^{-1}], and texture. Five replicates ($n = 5$) were used samples per test; additional details regarding the soil CEC, pH, TOC, and texture measurements are found in Supporting Information. TMX sorption coefficient (K_d) [L^3 M^{-1}] and sorption coefficient normalized to soil organic carbon content (K_{oc}) [L^3 M^{-1}] were determined via EPA method 835.1230 (USEPA, 2008a, 2008b) using composite soil samples collected from the two field locations. Soil physiochemical and hydraulic properties are shown in Table 1.

2.2. Column design and preparations

At each soil collection site, 12 m long by 0.3 m wide areas were excavated to a depth of 20 cm, exposing the subsurface B_t horizons. At the New Kent site, seventeen long (60 cm) and eight short (20 cm) columns were hand-packed using soil from the trench. All columns were made using 20 cm (inner diameter) Schedule 40 PVC Pipe. The 20 cm columns were packed with the A_p soil. For the 60 cm columns, the upper 20 cm and lower 40 cm were packed with the A_p and B_t soils, respectively. All fill soil was homogenized by hand and plant material and roots were removed. Soil layers were packed with a wooden piston (10 cm diameter) to match the measured in situ dry bulk density. The two column lengths were chosen to isolate the effects of the A_p and B_t layers.

At the Whitethorne site, a Giddings Rig was used to collect sixteen semi-intact cores (with 20 cm inner diameter PVC pipe) that were 60 cm in length, of which the lower 40 cm came from the extracted soil (moderate, fine, subangular, blocky structure). These columns therefore included soil structure within the B_t horizon, and are referred to as the "structured loam" treatment. In addition, an "unstructured" loam treatment was created using five 60 cm tall columns that were filled with sieved (<2 mm) and repacked B_t (20–60 cm) and A_p soil (0–20 cm) from the Whitethorne site. The upper 20 cm of all other loam columns, as well as ten additional 20 cm ("short") columns, were packed using the local A_p soil as described above. Column packing resembled in situ bulk densities, though some minor adjustments were made after packing, as detailed in the Supporting Information.

All columns were saturated with a 0.005 M $CaCl_2$ artificial rain solution for 48 h and left to drain completely for another 48 h prior to planting corn seeds (Kurwadkar et al., 2014; USEPA, 2008a, 2008b). This step was included to purge air-filled pores, force out large soil macrofauna, and remove background TMX (for more information on background TMX, see the supplemental information). After the columns stopped draining, three Cruiser Extreme® 1250 corn seeds (Syngenta; Greensboro, NC) were planted in each column to a depth of 4 cm (Roger et al., 2013); this amount corresponded to the recommended maximum yield planting density of 95,000 plants ha^{-1} (Lauer, 2009). Preliminary analysis revealed that each seed carried 0.6 mg of TMX.

2.3. TMX transport/leaching study

Forty columns ($n = 17$ for sand and $n = 23$ for loam) were chosen for the leaching study (Fig. S1). Within each soil texture class, columns were separated into a "with plant" treatment, where corn plants were allowed to grow, and a "no plant" treatment where seedlings were severed upon emergence to control for corn growth. This design was to understand the influence of the plant on the transport process. Each treatment was replicated 4–5 times by column lengths (20 cm and 60 cm) ($n = 4$–5). The five "unstructured" 60 cm loam columns were assigned the "with plant" treatment, for direct comparison to structured loam columns with viable corn. Soil columns were housed in a greenhouse at 24 °C, and exposed to 400-watt growth lights for 14 h and 10 min, daily. The hours of daylight were consistent with that of May 15th, 2015 in Blacksburg, VA, which was chosen to represent the median day length for the first month of the corn growing season (Brann et al., 2009; Straw, 2009).

The columns were watered every 3 days for a 30 day growing period with 300 mL (0.9 cm) of 0.005 M $CaCl_2$ solution each time. Volume and frequency of watering were chosen to reflect mean precipitation and return periods, based on Virginia precipitation data from 1952–2012 (UVA, 2015). The watering solution was applied at a rate of 70 mm hr^{-1} using a calibrated polyethylene cylinder (SERA-17, 2008). On Day 31, a large rainstorm event of 9 cm (3 L) was simulated, representing the mean daily maximum rainfall for March–April at the sites (UVA, 2015). Leachate was continuously collected from the bottom of each column. Samples were taken for analysis of TMX concentration and the collection containers were then emptied prior to each watering event (12 events total over 33 days). A simplified mass balance was then used to estimate cumulative evapotranspiration [L] by subtracting the amount of cumulative leachate from the cumulative simulated rainfall (as discussed in more detail in the Supporting Information). The daily evapotranspiration rate [L T^{-1}] was calculated by dividing the cumulative amount by the number of elapsed days (i.e., 33 days). This approach assumed that evapotranspiration was constant throughout the experiment, which was likely reasonable given the near constant temperature and humidity conditions in the greenhouse, particularly for the "no plant" columns.

2.4. TMX distribution and movement in soil

The remaining 33 columns were used to measure TMX uptake into corn plants and movement into surrounding soil. These columns were destructively sampled at corn stages V1 (8 columns; $n = 4$/soil texture), V3 (8 columns; $n = 4$/soil texture), and V5 (17 columns that were also used in the Leaching Study, as detailed in Fig. S1), which respectively corresponded to 8 19, and 33 days after planting. Corn growth stages were determined by counting corn "collars", e.g. stage V1 corresponds to one corn collar following the development of the first leaf (McWilliams et al., 2010). At growth stages V1 and V3, the sampled columns ($n = 4$/soil texture/growth stage) were cut longitudinally with a jig saw, destructively sampled, and measured for TMX concentrations. At growth stage V5, eight of the 60 cm "with plant" leaching columns, along with another nine "no plant" leaching columns ($n = 4$ for sand and 5 for loam), from the Leaching Study ($n = 4$/soil texture), were destructively sampled. Because TMX in plant tissue represented a minor contribution to the initial input from seed coatings (<0.5%), the current study reports TMX movement and distribution in soil. TMX was analyzed in rhizosphere, root soil, and in bulk soil (0–30 cm, 30–45 cm, and 45–60 cm depth intervals) (as discussed in the Supporting Information). Methods for collection of root soil and rhizosphere soil are described by Cushman (2017).

To focus on providing definitive evidence of any mobilization of TMX in the soil profile, TMX distribution in bulk soil was reported. The TMX measured in root soil and rhizosphere soil were grouped together as a lumped average deemed the "plant-associated" TMX. This grouping allowed for contrast between soil that is associated with plant tissues versus that which is in the bulk soil, and thus assumed to be environmentally available. In the case of the "no plant" treatments, the "plant-associated" TMX consisted only of soil associated with seed and decayed root tissue.

Table 1
Soil physiochemical properties.

Soil	Depth (cm)	Texture	Sand (%)	Silt (%)	Clay (%)	Bulk density (g cm^{-3})	Porosity (%)	TOC (%)	pH	CEC (meq 100 g^{-1})
Loam A_p	0–20	Loam	38.7	43.6	17.7	1.37 ± 0.093	49	1.12*a*	5.8	$4.7 \pm 0.15a$
Loam B_t	20–60	Loam	33.4	40.1	26.4	1.64 ± 0.130	39	0.3*c*	5.9	$4.5 \pm 0.75a$
Sand A_p	0–20	Loamy sand	85.9	7.69	6.41	1.52 ± 0.108	44	1.46*b*	4.8	$3.5 \pm 0.50b$
Sand B_t	20–60	Fine sand	87.4	9.91	2.71	1.67 ± 0.070	38	0.49*c*	4.1	$1.8 \pm 0.33c$

Different letters denote significant differences ($p \le 0.05$). Bulk Density and Cation Exchange Capacity (CEC) are expressed as mean ± standard deviation.

2.5. Statistical analysis

TMX concentrations and mass recovered in the soil profile were analyzed using two-way ANOVA. These tests were conducted on log transformed data by bulk soil depths of 0–30 cm and 30–45 cm, and rank transformed data for the 45–60 cm section. Rank transformation was used as a way to transform data to normality and provided an avenue for parametric factorial comparisons (e.g. two-way ANOVA), which is the functionally equivalent to traditional non-parametric tests (Conover, 2012; Conover and Iman, 1981). For soil analysis, texture (sand and loam) and corn stage (V1, V3, V5, and a combined V5-no plant) were treated as factors. The V5-no plant combination was created to identify the corn plant's influence on TMX distribution. Two-way ANOVAs were tested on TMX concentration in final leachate, TMX mass transport data, and total leachate volume, where texture and plant influence (plant and no plant) were treated as factors. TMX transport data were rank transformed to normality for 60 cm columns and log transformed for 20 cm columns. A one way Kruskal Wallis test was also conducted to compare TMX transported in structured versus unstructured loam treatments. Normality was determined via visual inspection of histogram and normal quantile plot results. Homogeneity of variances was confirmed via Fligner's test. Tukey's multiple comparisons test was run on all resulting ANOVA results. All statistical tests were conducted in R version 3.2.2 with $\alpha = 0.05$.

3. Results and discussion

The saturated hydraulic conductivity (K_s) measurements from the soil cores showed that the loam soil had much higher variability, both between the A_p and B_t horizons and between samples within each horizon (Fig. 1). The median K_s value for the loam A_p horizon was 860 cm d^{-1}, which was approximately two times greater than the median K_s value for the sand A_p (410 cm d^{-1}). The median K_s values for the loam B_t horizon, on the other hand, was 36 cm d^{-1}, which was nearly a factor of 10 less than the K_s value for the sand B_t horizon (300 cm d^{-1}). However, individual cores within the loam B_t horizon showed K_s values as high as 1100 cm d^{-1}, which exceeded all values measured for the sand B_t horizon. Taken together, these K_s estimates show that the loam soil has

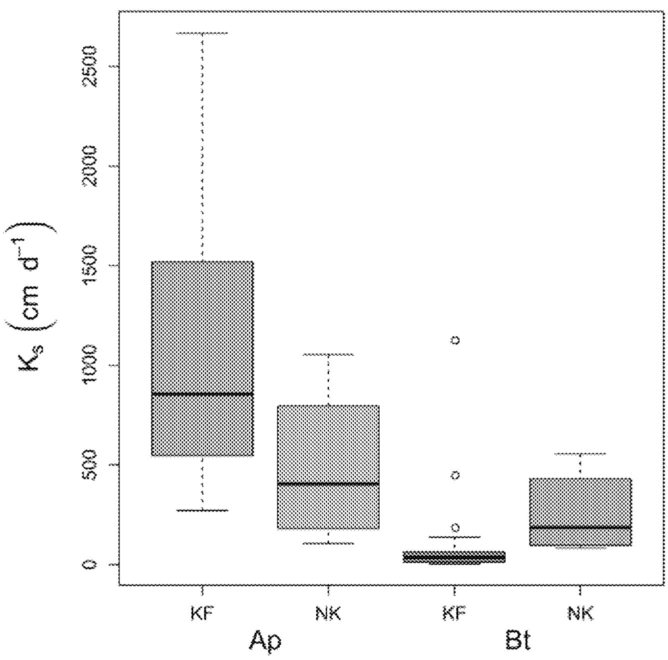

Fig. 1. Saturated hydraulic conductivity (K_s) values (cm d^{-1}) measured for the upper Ap and lower Bt horizons from the two studied soils (KF = Kentland Farm "loam" soil; NK = New Kent "sand" soil). $n = 17$ for KF/A_p; $n = 12$ for NK/A_p; $n = 21$ for KF/B_t; $n = 8$ for NK/B_t.

greater variability in its hydraulic conductivity, which we hypothesize to be an indication of preferential flowpaths, for example due to soil structure or connected biopores. We also hypothesize that the unstructured loam columns will lack these preferential pathways due to the repacking process, and that the hydraulic conductivity of those columns will be better represented by the lowest, rather than the highest, K_s readings.

TMX leaching was minimal in all treatments for the first 30 days of the experiment; however, TMX was detected in leachate for all columns following the final (9 cm) precipitation event on day 31 (Fig. 2). Texture played a significant role in TMX transport through the 20 cm columns (two-way factorial ANOVA, $p = 0.008$), as sand columns transported higher concentrations of TMX during the final leaching event compared to the loam columns (sand, with plant vs loam, with plant; Tukey, $p = 0.03$; Fig. 2a). No significant differences were found between treatments for total drainage and total TMX mass transported, and plants showed no significant effect on TMX leaching in terms of mass transport, final leachate concentration, and drainage in 20 cm columns (two-way factorial ANOVA, Tukey, $p > 0.05$; Fig. 2a and b). All 20 cm columns leached TMX at concentrations above the United States Environmental Protection Agency (EPA) benchmark for acute toxicity to aquatic invertebrates (Anderson et al., 2013) (Fig. 2a). This finding suggests that surface soil horizons in agricultural systems may be capable of transporting harmful concentrations of TMX, particularly during more intense/longer duration rainfall events.

Contrary to the 20 cm columns, in the 60 cm columns the structured loam column soil facilitated greater TMX transport than the sand, with greater cumulative mass leaching over the course of the experiment (Fig. 2f), and higher TMX concentrations during the final 3 L irrigation event (two-way factorial ANOVA, $p < 0.001$; Fig. 2e). Viable corn plants had no effect on TMX transport in terms of mass or concentration (two-way factorial ANOVA, $p > 0.05$). Soil texture affected the total leachate volume; however, the difference was attributed to a higher volume of leachate in loam columns without viable plants compared to sand columns with plants (Two-Way factorial ANOVA, $p = 0.006$; Tukey, $p = 0.007$; Fig. 2f). Soil structure also had a significant effect, as the structured loam columns transported more TMX mass, contained a higher concentration in final leachate, and yielded a higher drainage volume than the unstructured loam columns (Kruskal-Wallis, $p = 0.03$, $p = 0.007$, $p = 0.03$, respectively; Fig. 2c and d). It should also be noted that even unstructured loam transported TMX at higher concentrations in the final leachate than the sand columns (unstructured loam, 1.65 ± 1.96 μg L^{-1}; sand, 0.18 ± 0.10 μg L^{-1}; both containing viable plants), possibly due to the sand having high near-surface evapotranspiration over the course of the experiment (see ET Results and discussion section below).

In the soil distribution study, TMX was detected in the 0–30 cm bulk soil (outside of rhizosphere and root soil) as early as 8 days after planting (i.e., the V1 growth stage), with average concentrations as high as 75 μg kg^{-1} (sand columns at V1 corn stage; Fig. 3a). The appearance of TMX in bulk soil was complemented by migration of the pesticide from plant-associated soil (plant-associated soil: Fig. 4; bulk soil: Fig. 3). By the V5 corn stage (33 days after planting), TMX had moved into the 30–45 cm and 45–60 cm soil sections in significant concentrations (Tukey, $p < 0.001$), though the majority of TMX still remained in the 0–30 cm bulk soil. Corn stage showed a significant effect on TMX concentration and recovered mass for all depth intervals (two-way factorial ANOVA, $p < 0.001$). TMX concentrations in bulk soil were generally higher in the sand compared to the loam soil; however, texture only significantly affected TMX distribution in the 0–30 cm section at the V5 corn stage (two-way factorial ANOVA, $p < 0.001$; Tukey, $p < 0.001$; Fig. 3a). Further, using the experimentally determined sorption coefficient from sand columns ($K_d = 0.5$ L kg^{-1}), the liquid phase concentration could be as high 150 μg L^{-1}, which exceeds the EPA's aquatic life benchmark of 17.5 μg L^{-1} by nearly an order of magnitude (Anderson et al., 2013). This predicted liquid phase concentration was within a factor of two of the concentration measured in the leachate from 20 cm tall sand

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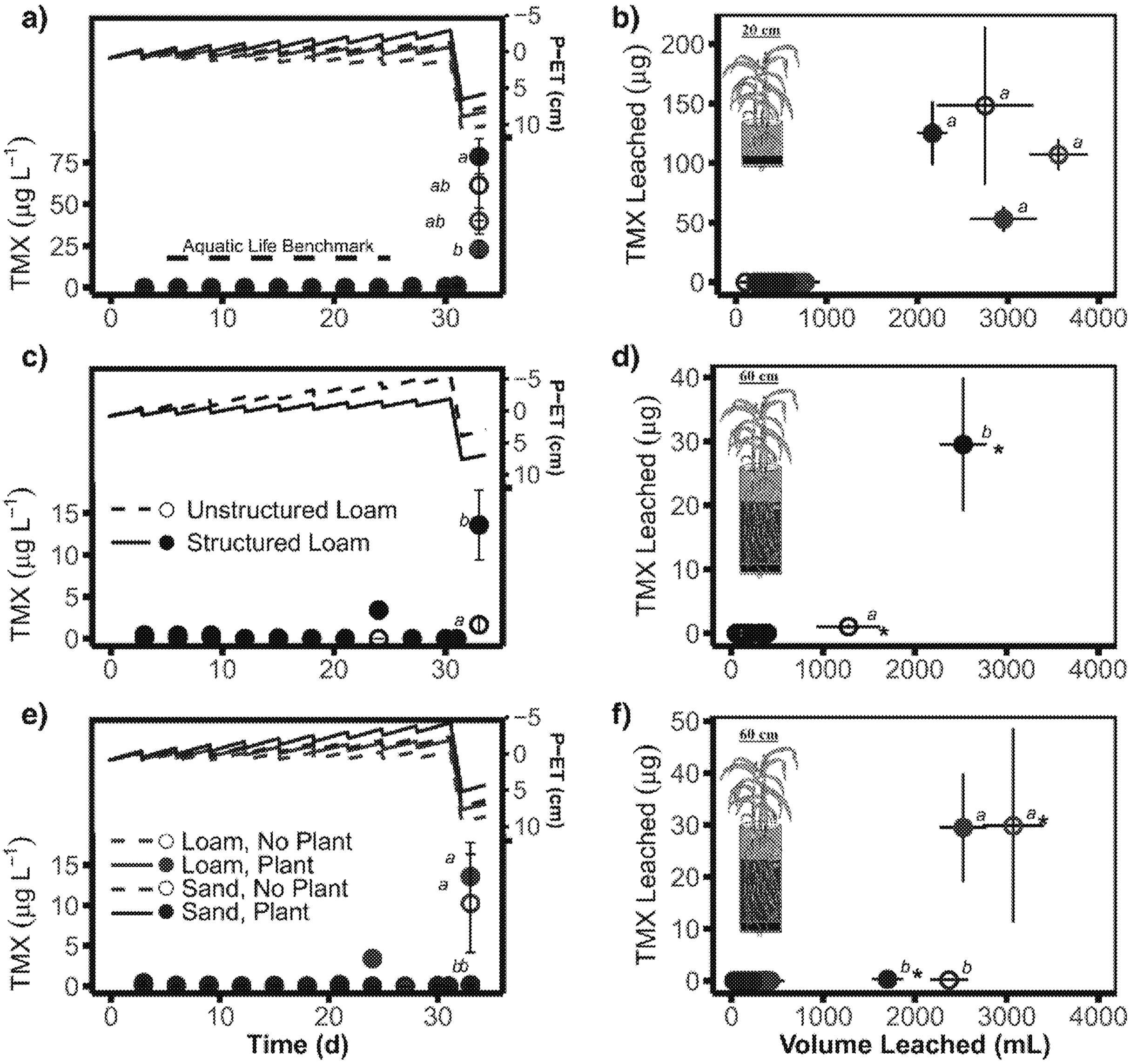

Fig. 2. TMX concentration in leachate vs time with Cumulative Rainfall/Cumulative Evapotranspiration Deficit (P – ET) (left), and mass of TMX transported vs cumulative drainage (right). Column-plant graphics specify the column size for that row of panels (e.g. first row depicts 60 cm column results). *Different letters* denote significant differences for Y variable, while (⁎) designates differences along the x axis ($p \leq 0.05$). Error bars represent standard error. Missing SEs indicate $n = 1$ column at time of measurement. The dashed black line in a) represents the Environmental Protection Agency benchmark for acute toxicity to aquatic invertebrates (17.5 μg L^{-1}) (Anderson et al., 2013).

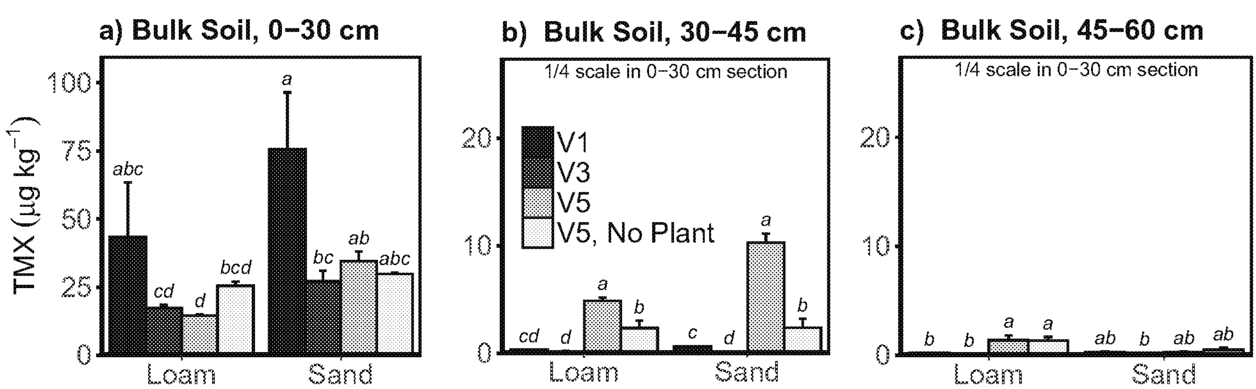

Fig. 3. TMX concentration detected in bulk soil fractions 0–30 cm a), 30–45 cm b), and 45–60 cm c) over three corn stages (V1, V3, and V5). Error bars represent SE and *different letters* represent significant differences ($p \leq 0.05$). Y axes in 30–45 cm and 45–60 cm fractions are 25% that of the 0–30 cm section.

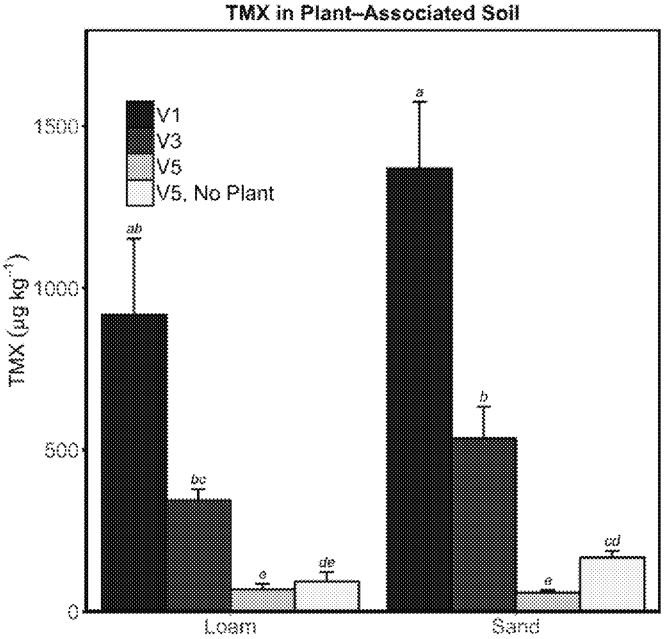

Fig. 4. TMX concentration detected in plant-associated soil (lumped average between root and rhizosphere soil). V5 No Plant treatment represents seed soil (see description in Materials and methods section). Error bars represent SE and *different letters* represent significant differences ($p \leq 0.05$.

columns (e.g. 75 μg L^{-1}), showing that the K_d value determined in the laboratory from small-scale samples was applicable to the larger-scale columns.

Because sandy soils typically have larger, less reactive particles and higher hydraulic conductivity than finer-textured soils, it is often assumed that coarse-textured soils will transport more solutes (e.g. pesticides) than finer-textured particle soils (Katagi, 2013; Mallants et al., 2011). This assumption is supported by the measured soil physiochemical (compared with loam, sand had lower measured K_d of 0.5 L kg^{-1} and K_{oc} of 57.9 L kg^{-1}; also lower measured CEC value of 1.8–3.5 meq 100 g^{-1}) and hydraulic (e.g., the sand produced higher median K_s values than the loam in the B_t horizons; Fig. 1) properties, as well as by the TMX mobilization observed in the 20 cm sand columns (Fig. 2a and b). However, in the 60 cm columns the loam soil was associated with greater TMX transport (Fig. 2e and f) and less retention than the sand (Fig. 3) despite its physiochemical properties appearing to favor more pesticide retention relative to the sand (compared with sand, higher measured K_d of 0.8 L kg^{-1} and K_{oc} of 127 L kg^{-1}; also larger measured CEC value of 4.5–4.7 meq 100 g^{-1}). Higher levels of the pesticide leaching from structured loam columns (compared to unstructured loam; Fig. 2c and d) and greater variation in measured K_s in structured loam columns (Fig. 1) suggest that pore heterogeneity and preferential flow (Øygarden et al., 1997) may have driven TMX transport. For example, the cumulative mass of leached TMX was more than two orders of magnitude higher in the structured loam soils than the sand (e.g., 29.9 μg vs 0.17 μg for the no plant treatments; Fig. 2f). This trend was apparent with and without the presence of viable plants, and suggests that structural flow pathways located in the intact loam B_t horizon may have become activated during rain events. This process likely then, allowed for downward advection of TMX through the profile. In this experiment soil structure was thus seen to be a more important control on TMX transport than soil texture, which has several implications. For one, using repacked columns for transport studies may prove inadequate for representing well-structured soils, and such experimental setups may under-predict neonicotinoid movement. Also, the adoption of "soil health" building agricultural practices such as no tillage and cover crops, which provide greater soil macroporosity and preferential flow, may be unexpectedly increasing the likelihood of pesticide transport (Alletto et al., 2010).

Further, the significantly higher evapotranspiration estimates shown in 60 cm sand columns (loam, with plant vs sand, with plant; Table 2c), coupled with higher observed concentrations of TMX in the soil profile (sand vs loam; Fig. 3), suggest that evapotranspiration may have concentrated much of the TMX at or near the soil surface, in a process referred to here as "evapo-concentration." The loam soils also likely experienced some evapo-concentration, though the effect was more muted in the structured loam columns (i.e., more total TMX leached by the end of the experiment even though total leachate amount only differed statistically between the structured loam with no plants and sand with plants; Table 2) and was more pronounced in the unstructured 60 cm loam columns, which showed the greatest amount of evaporation (Table 2). By comparison these results suggest that macropore networks within structured soils may reduce evaporation of water and evapo-concentration of soluble pesticides like TMX. These reductions may result from a combination of two processes: 1) leaching via preferential flow can remove water and soluble compounds from the system altogether, and 2) macropores can limit the ability of water to move back up towards the drying front via capillary rise, due to the relatively low tensions of water held in those pores. Also, in nearly all cases the columns with viable plants yielded less drainage water (thus displaying higher estimated ET; Fig. 2 and Table 2) and transported less TMX mass (excluding 60 cm sand columns) than the equivalent "no plant" columns (Fig. 2), though the differences were not significant. Still, these observations indicate that evapo-concentration (aided by viable plants) likely retarded any noticeable downward migration of TMX so long as cumulative ET exceeded cumulative precipitation, P (i.e., $P - ET < 0$).

Corn plants influenced the distribution of TMX in the 30–45 cm bulk soil section (two-way factorial ANOVA, $p < 0.05$; Fig. 3b), which can be seen as higher concentrations detected in columns containing viable plants compared to those controlled for plant growth in the V5 corn stage (i.e., after 33 days of growth). Corn plants appear to have amplified the downward mobilization of TMX (Fig. 3), which may be linked to a more massive and extensive root network (Fig. 5) present in the V5 corn stage. Higher concentrations of TMX were detected in plant-associated soil for columns without viable corn plants at V5 (sand; Tukey, $p < 0.01$; Fig. 4), which further illustrates the plant-assisted movement of this compound; however, this observation may be partially attributed to accelerated decomposition of TMX in the presence of root exudates (Akbar and Sultan, 2016). Altogether, root-facilitated preferential transport of TMX exceeded preferential retention of TMX via root water uptake or localized evapo-concentration. As such, living plant roots may provide a previously unrecognized conduit for pesticide leaching, though it is not yet known if this process is relevant in field settings.

TMX concentrations found in the near-surface bulk soil were sufficiently high to pose environmental threats. For example, given the 75 μg kg^{-1} concentration measured in the sand columns at the V1 growth stage, only 0.5 g of that soil would contain enough pesticide to kill 50% of any given honeybee population (Anderson et al., 2013; Sanchez-Bayo and Goka, 2014). Likewise, the leachate from the 20 cm tall columns (which represents the mobile compounds in the near-surface soil) had measured concentrations as high 75 μg L^{-1}, which exceeds the EPA's aquatic life benchmark of 17.5 μg L^{-1} by nearly a factor of five (Anderson et al., 2013). TMX concentrations at depths below 30 cm may also be of concern. For instance, the 30–45 cm bulk soil in the sand columns with viable plants showed TMX concentrations as high as 10 μg kg^{-1} TMX at corn stage V5, which translates to 20 μg L^{-1} (using the experimentally-determined sorption coefficient of $K_d = 0.5$ L kg^{-1}). This value again exceeds the EPA benchmark and is sufficiently elevated to induce tissue necrosis to some aquatic invertebrates (Uğurlu et al., 2015).

The maximum concentration in the 30–45 cm bulk soil occurred nearly 20 days after the maximum concentration was detected in the 0–30 cm section, indicating that TMX mass was migrating downward in response to percolating water. Thus, while the leachate from the

Table 2
Estimated cumulative evaporation (ET) and average daily ET rate in: a) Small (20 cm) columns; b) Tall (60 cm) columns from soil structure study; and c) Tall (60 cm) columns without an unstructured loam treatment.

	Texture	Column size (cm)	Plant influence	Structure	Cumulative ET (cm)	ET rate (cm d^{-1})
a)	Loam	20	Plant	*NA*	11 ± 2.3*a*	0.32 ± 0.035
	Loam	20	No plant	*NA*	8.7 ± 2.6*a*	0.26 ± 0.069
	Sand	20	Plant	*NA*	13 ± 0.92*a*	0.40 ± 0.028
	Sand	20	No plant	*NA*	11.3 ± 3.4*a*	0.34 ± 0.10
b)	Loam	60	Plant	Structured	12 ± 2.1*b*	0.36 ± 0.024
	Loam	60	Plant	Unstructured	16 ± 1.4*a*	0.48 ± 0.067
c)	Loam	60	Plant	*NA*	12 ± 2.1*b*	0.36 ± 0.024
	Loam	60	No plant	*NA*	10 ± 2.1*ab*	0.31 ± 0.064
	Sand	60	Plant	*NA*	14.6 ± 1.07*a*	0.44 ± 0.032
	Sand	60	No plant	*NA*	12 ± 1.3*ab*	0.37 ± 0.040

Values are expressed as mean ± standard deviation and *different letters* denote significant differences ($p \leq 0.05$). *NA* is used to distinguish the soil structure study from other soil columns. Section **a)**, row 1 and **b)**, row 1 are equivalent, as they depict the same soil column.

60 cm sand columns had only trace amounts of TMX by the end of the experiment (Fig. 1e; 1f), continued rainfall (or lower ET) would have likely led to deeper migration and eventual leaching of the pesticide.

This experiment provided a conservative simulation of TMX mobilization in soils (e.g. low rainfall, repacked A_p and some B_t horizons), whereas field conditions could potentially favor even greater mobilization and transport of this compound. For example, greenhouse conditions (e.g., constant air circulation and the use of growth lights) likely enhanced evapotranspiration rates compared to field settings, resulting in greater evapo-concentration (as seen in Table 2 and in the P – ET estimates shown in Fig. 2). In addition, agricultural systems often are subjected to one or more large-scale rainfall events throughout the growing season, (Kunkel et al., 2013) rather than a consistent regiment of small-scale events (e.g., the 0.9 cm of rainfall applied every three days) that was applied in this study.

3.1. Implications and conclusions

Seed coatings account for ~60% of the global product use (Goulson and Kleijn, 2013). However, no previous study has definitively proven that neonicotinoids, once planted, can become transported from crop seed coatings. This study establishes that neonicotinoid can be transported in concentrations considered acutely toxic to aquatic life. If similar conditions exist in field soils that have well-connected macropore (e.g. the structured loam in this study) and/or tile-drain networks, then TMX could reach ground water and surface water systems at potentially harmful concentrations. These results also imply that plant growth and development may enhance neonicotinoid leaching, as the preferential transport along roots and root channels appear to have exceeded the ability of the plants to systematically uptake and retain TMX. This effect may become even more pronounced during short, high-intensity rainfall events, during which time neonicotinoid compounds can become mobilized via rapid preferential flow.

Even in the absence of preferential flow, TMX may become leached from soils over time. Given that the half-life of TMX can exceed 350 days, (Goulson and Kleijn, 2013), the compound could persist in fields during the growing season (i.e., when evapo-concentration would be greatest) and then become leached during the non-growing season (i.e., when evapo-concentration conditions would diminish). Future neonicotinoid application rates may also increase in response to greater pest pressures (for example driven by changes in climate conditions and weather variability; Koleva and Schneider, 2010), which could further increase concentrations and mobility of these compounds in the environment. Based on these findings, we expect that this study will serve as a reference for environmental risk assessment of neonicotinoids and a serve as material for pesticide transport modeling.

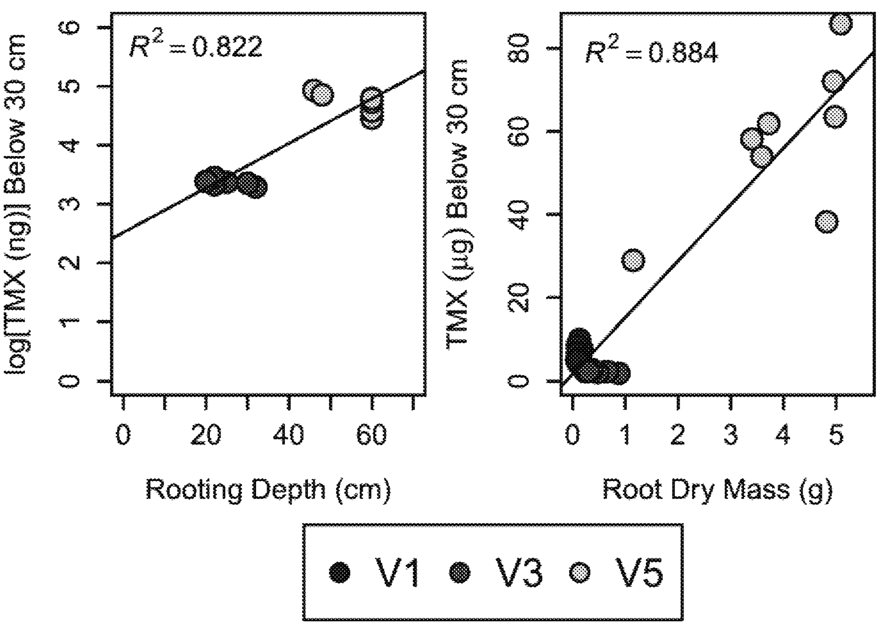

Fig. 5. Linear relationship between: the mass recovered below the 0–30 cm bulk soil section, rooting depth (left; log transformed), and root dry mass (right). Root length was not recorded for V1 columns.

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Appendix A. Supplementary data

TMX background concentrations in soil, details regarding the column design, soil characterization, experimental design, analytical approach, TMX mass recovered bulk and plant associated soil, estimation of evapotranspiration, and details to statistical analysis of TOC, CEC, plant-associated soil and ET results can be found in the Supporting Information. Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.scitotenv.2017.11.031.

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